Remarks

Claims 1-29 are pending. Claims 16-24 and 26 are withdrawn, and claims 1-15, 25 and 27-29 are under examination. Claims 1, 11, 12, 13 and 14 are amended.

Claim 1 has been amended to include the feature of claim 7 and to include a reference to derivatives of the signal peptide. The derivatives of the claim are derivatives which have at least 40% sequence homology to the signal peptide and which retain the ability of the signal peptide to enhance or induce secretion of the target protein. Support for this definition of the derivatives of signal peptides can be found on page 9, line 21 to page 10, line 3. In addition, to improve the clarity of the claim, the claim has been restructured slightly.

Claims 11 and 12 are amended to correct the obvious inadvertent misspelling of "Gaussia."

Claim 13 has been amended to delete reference to fragments, and the reference to derivatives has been qualified in the same way as the reference to derivatives in claim 1.

Claim 14 has been amended to define the recited fragments in terms of their length. Support for this is given on page 12, lines 28 and 29. The variants of the claim have been defined structurally in the same way as the reference to derivatives in claim 1, and the functional definition has been inserted so as to be applicable to the variants and the fragments of this claim.

No new matter is believed added by these amendments. Thus, their entry and consideration are respectfully requested.

Errors Introduced During Publication

Applicants note the following errors in the published version of the present application that were not present in the application as filed or introduced by any amendment:

[0036] The invention utilizes signal peptides from bulk-secreted proteins. Preferred are signal peptides from a copepod or an ostracod, e.g. Gaussia ~~princess~~princeps or Vargula hilgendorfii. Particularly

preferred are the signal peptides from Gaussia (MGVKVLFALICIAVAEA; SEQ ID No. 1) or Vargula (MKIILSVILAYCVT; SEQ ID No. 2) luciferase and most particularly the signal peptide from Gaussia luciferase.

[0078] In a particularly preferred embodiment the invention provides a method of producing a target protein, which method comprises expressing said protein in a mammalian host cell which contains a nucleic acid molecule which encodes a chimeric protein, said chimeric protein comprising a signal peptide from a non-mammalian bulk-secreted protein and said target protein. Preferred are signal peptides from a copepod or an ostracod, e.g. Gaussia ~~princess~~princeps or Vargula hilgendorfii. Particularly preferred are the signal peptides from Gaussia or Vargula luciferase and most particularly the signal peptide from Gaussia luciferase.

Corrections to these above paragraphs are indicated using strikethrough for deletions and using underlining for additions. Furthermore, claim 12 of the published application also shows a misspelling of "princeps" that was not in claim 12 as filed and was not introduced by any amendment. Applicants have not submitted any amendment to address the obvious errors on the part of the Patent Office. If any additional action is required on the part of applicant to address these errors on the part of the Patent Office, applicants would appreciate notification of same.

Drawings

The drawings are objected to because Figure 1 discloses amino acid sequences without SEQ ID NO: identifiers. The Office Action suggests either identifying the SEQ ID NOs for the respective sequences in the figures or in the brief description of the drawing section of the specification. Corrected drawing sheets in compliance with 37 CFR 1.121 (d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the

appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121 (d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

In response, applicants provide a Replacement Sheet for Figure 1 that includes sequence identifiers. Applicants also have amended the description of Figure 1 to include sequence identifiers. No new matter is added by these amendments, and their entry is respectfully requested.

Objection to the Specification

The disclosure is objected to because there is no section identified as brief description of the drawings. Suggest identifying the brief description of the drawing section.

In this regard, the specification has been amended to insert a section heading before the description of the drawings. Thus, this objection is believed to be overcome and its withdrawal is respectfully requested.

Rejections under 35 USC § 121, second paragraph

Claims 1-15 and 27-29 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In this regard the Office Actions states the following:

> The term "majority" in claim 7 is a relative term which renders the claim indefinite. The phrase "of the native protein" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. It is unclear how much of the native protein can be included within the chimeric protein.

The feature of claim 7 which specifies that the chimeric protein does not incorporate the majority of the native protein of the signal peptide has been introduced into claim 1, and claim 7 has been cancelled. With regard to this limitation in claim 1, it is submitted that it is clear from the description of the invention in the application as a whole that the chimeric protein is to include the signal peptide from a bulk secreted protein and that the remainder of peptide sequence of the bulk secreted protein is the native protein and is largely inconsequential to the functioning of the invention. However, it is also clear to the skilled person that some remnant of the non-signal peptide sequence of the bulk secreted protein is not necessarily a hindrance to the chimera either and so some of the non-signal peptide sequence of the bulk secreted protein can be incorporated in the chimeric protein. Of course, the skilled man would know that this superfluous sequence should ideally be kept to a minimum, i.e. the chimeric protein would not incorporate the majority of the non-signal peptide sequence of the bulk secreted protein.

On a more fundamental level, the term "majority" can be taken in its normal dictionary meaning, i.e. "more than 50%". In the present context then, the feature of claim 7 (now incorporated in amended claim 1) can be considered to mean that the chimeric proteins of the claims do not incorporate any more than 50% of the sequence of the native protein, although preferably the chimeric protein will have as little as possible of the non-signal peptide sequence of the bulk secreted protein.

Based on the teaching of the application, and the dictionary, the meaning of this term would be understood by the skilled person such that the metes and bounds of the claim are understood. In contrast, the Office has provided no basis to believe any differently. In the absence of any such basis, the present basis for rejection is unsupported and should be withdrawn.

The Office Action further states the following:

> In claim 27, it is unclear how the signal peptide is from the bulk-secreted protein and the target protein. It appears the signal peptide should be from the taxonomically distinct bulk-secreted protein.
>
> The term "bulk" in claims 1 and 27 is a relative term which renders the claim indefinite.
>
> The term "secreted protein" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. What amount of a secretion is encompassed by a bulk-secreted protein?

To clarify that the signal peptide referred to in claim 27 is the signal peptide from the bulk secreted

protein and is not the signal peptide from the target protein, the claims have been restructured to clearly separate the two basic components of the chimera. For consistency, similar amendments have been made to claim 1.

The Examiner has concerns with the clarity of the term "bulk secreted". This term is defined in detail in the specification (paragraph bridging pages 8 and 9) and is further described on pages 6 and 7 in relation to specific examples. It is submitted that the term is clear to the skilled person on the basis of this discussion alone. Moreover, the skilled person would understand the term from the common general knowledge in the art.

In support of this position, enclosed are documents by Perrin *et al.*(Attached as Exhibit 1), Liu *et al.* (Attached as Exhibit 2), Kolarow *et al.* (Attached as Exhibit 3) and Lessmann *et al.* (Attached as Exhibit 4) in which the term "bulk secreted" is used. These documents date from 1992 to 2007 and are all articles from well respected, peer reviewed, scientific journals. As such, the use of the term would not have been permitted if it were unclear. In addition, the Lessmann paper is a review article that summarizes the work in the field and can be assumed to be reflective of established terminology in the field. This document uses the term "bulk secretion" to describe the transient release of large amounts of proteins from cells in culture and neurons upon response to transient extracellular stimulation (i.e. the definition of bulk secretion used by the present inventors). The Examiner is directed to Sections 3 and 4 of Lessmann *et al.*, the abstract of Liu *et al.*, the first two full paragraphs on page 10359, right hand column of Kolarow *et al.* and the entire disclosure of Perrin *et al.* in which the terms are used numerous times. The Perrin article uses the term very often, inter alia in the abstract, the legends to Tables I and II and the first paragraph of the right hand column of page 125.

In summary, the term "bulk secreted" is a term that has been and still is in use in the technical field and so is understandable by the skilled person. The skilled person will therefore know of proteins that are bulk secreted, would know how to identify proteins that are bulk secreted and would also easily be able to test, on the basis of simple and routine techniques (e.g. microscopy, immunoassay, etc.) whether a particular protein is bulk secreted. Identification of the signal peptides from such proteins is also entirely routine using standard molecular biology techniques and computer modelling programs. As described herein and as evidenced in the literature, the skilled man would know precisely what is encompassed by this term and how to determine whether a particular protein is a bulk secreted protein or not. In contrast, the Office has

provided no basis to believe any differently. In the absence of any such basis, the present basis for rejection is unsupported and should be withdrawn.

Rejections under 35 USC § 112, first paragraph

Claims 13 and 14 are rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement. In this regard, the Office Action states the following:

> The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The claims are rejected for failing to describe a representative genus of fragments, derivatives, and variants of the SEQ ID NOs encompassed by the claims. The disclosure does not provide sufficient structure to function correlation for the genus of fragments, derivatives, and variants thereof that will maintain the signal sequence function.

The Examiner has objected to the references to fragments and derivatives/variants in claims 13 and 14. Claims 1 and 27 now also refer to derivatives. However, these derivatives have been strictly defined structurally by reference to minimum percentage sequence homology and functionally with an accompanying functional limitation. A reference to fragments has not been used in claims 1 and 27 as amended and has been deleted from claim 13. Claim 14 does retain reference to fragments, but those fragments are also defined very closely in both structural and functional terms. These limitations are described at length in the description (pages 9 to 12). Accordingly, it is submitted that the fragments and derivatives/variants of these claims are readily identifiable and the properties thereof and how they compare with the properties of the unmodified signal peptide are readily testable using routine workshop protocols (e.g. microscopy, immunoassays, etc.) on the basis of the written description and the common general knowledge in the art. As such one of skill in the art would recognize that applicants invented the subject matter claimed, i.e., the fragments and derivatives as specifically claimed.

In support of this there is recited below a table that shows the results of an experiment that tests a number of substitution mutants of a bulk secreted protein, Oikosin 1. Oikosin 1 is produced by the marine tunicate, Oikopleura dioica. In this experiment the ability of Oikosin 1 signal peptide (MLLLSALLLGLAHGYS) to drive the secretion of Gaussia luciferase protein was measured and substitution mutants (i.e. derivatives/variants) at one or more of the three positions (residues 4, 5 and 13; L, S and H) were assessed for their relative abilities. As will be seen from the data below, various substitution mutations can be made which retain at least 82% of the activity of the reference sequence and can even have

up to 246% of the activity of the reference sequence.

Signal peptide sequence at residues 4, 5 and 13 respectively (wild type = (LSH))	Oik1(LSH)	**QYH**	**VPH**	**LPH**	**RIH**	
Luciferase activity in medium (% of activity with wild type signal peptide)	100	89	126	142	188	
Signal peptide sequence at residues 4, 5 and 13 respectively (wild type = (LSH))	Oik1(LSH)	**LSP**	**LSI**	**LSM**	**LSC**	**LSF**
Luciferase activity in medium (% of activity with wild type signal peptide)	100	89	185	191	224	246
Signal peptide sequence at residues 4, 5 and 13 respectively (wild type = (LSH))	Oik1(LSH)	**VLL**	**SFL**			
Luciferase activity in medium (% of activity with wild type signal peptide)	100	201	222			

It can be seen that mutations in the sequence of a signal peptide from a bulk secreted protein can readily be made and tested for activity relative to the reference sequence. In this particular example the property of the target protein to emit light under certain conditions was conveniently used as the reporting mechanism. However, the levels of target protein can be detected by any convenient means appropriate for the identity of the target protein. Indeed, most likely there will be antibodies against the chosen target protein which can conveniently be used in systems such as an ELISA system to detect relative concentrations of the target protein in any particular sample.

From the above discussion it is clear that from the written description and the common general knowledge the skilled person would readily be able to identify bulk secreted proteins and he would readily be able to identify the sequence of the signal peptide. He would also readily be able to prepare derivatives of that signal peptide sequence and test the activity of that sequence relative to the reference peptide. Because of the state of the art regarding signal sequences and their modification, the defined fragments or derivatives of the defined signal sequences would be viewed as in the possession of applicants. It is submitted therefore that the fragments/derivatives/variants of the claims as amended do find adequate written description in the application as filed.

Rejections Under 35 USC § 102

Claims 1, 4-7, 9, 15, and 25 are rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Allet *et al.* (Protein Expression and Purification 9:61-68 (1997); Previously cited). In this regard, the Office Action further states the following:

> Allet et al. disclose the use of a bacterial signal peptide sequence to direct efficient secretion of eukaryotic proteins in Baculovirus expression system. Allet et al. disclose the use of signal sequence from Staphylococcal protein A fused with eukaryotic proteins for expression in Baculovirus insect expression cells (see page 62-63, Materials and Methods section). The signal sequence was 36 amino acids in length (see page 65, Figure 2). Figures 4 and 5 show the purification of fusion proteins from expression system (see page 66).

As the Examiner points out, Allet *et al* discloses use of the signal peptide sequence from Staphylococcal Protein A to drive secretion of eukaryotic proteins in baculovirus insect expression cells. Staphylococcal Protein A is well known to be a protein that is found in the cell wall of *Staphylococcus* bacteria. Accordingly, it is not a secreted protein and certainly not a bulk secreted protein as defined in the application as filed and described above. As such, it fails to disclose the elements of the present claims, and so the claims are novel over this document. Withdrawal of this rejection is believed to be merited and is respectfully requested.

Claims 1-7 and 15 are rejected under 35 U.S.C. 102(a) as allegedly being anticipated by Brumell *et al.* (Traffic 4:36-48 (Jan. 2003)). In this regard the Office Action further states the following:

> Brumell et al. disclose a SopD2 protein that is a novel type III secreted effector of Salmonella typhimurium that targets late endocytic compartments upon delivery into host cells. Brumell et al. disclose the synthesis of N-terminal truncated SopD2 fusion proteins with GFP. Brumell et al. disclose the transfection of mammalian HeLa cells with SopD2-GFP chimeric proteins, wherein the SopD2 portion provides a signal sequence (see page 43, Figure 3, and Materials and Methods section, Cell culture section).

The disclosure of this document can be distinguished from the presently claimed invention on a number of points.

SopD2 is a protein from *Salmonella typhimurium* that has the property of being able to translocate from the bacteria into mammalian cells. This is via a Type III secretion system. This system is considered distinct to vesicle-based secretion processes of which one example is the bulk secretion system. In the Type

III secretion system the protein to be transported is translocated directly from the bacteria into the cytoplasm of the eukaryotic host by proteinaceous transporters. There is no release into the extracellular media or extracellular space nor are vesicles used at any point in the process. In the Type III system the transporters recognise individual proteins to be transported in the cytoplasm of the bacteria and association between the proteins to be transported and the transporter results in the movement of the cargo from the bacteria cytoplasm to the cytoplasm of the host cell. It is not a system where vesicles containing the cargo fuse with the outer membrane of the cell and release their contents from the cell. The use of the term "secretion" in this context is perhaps misleading: "transport" is perhaps more accurate. The Examiner is directed to the last full paragraph on page 8 and the paragraph bridging pages 8 and 9 of the application as filed for a description of vesicle based secretion systems (e.g. bulk secretion). Clearly this is a fundamentally different system to a Type III secretion system.

These fundamental differences are reflected in fundamental differences in the structural properties of the "signal" sequences of Type III secreted bacterial proteins and the signal peptides of proteins that are secreted from cells using vesicle-based systems. Because Type III secretion is protein mediated and not vesicle mediated the "signal" in Type III protein is a part of the mature protein. It is this portion that is recognised by the Type III transport machinery. In contrast, a signal peptide is transiently a part of a vesicle-secreted protein and is removed once that nascent protein is directed to the vesicle packaging pathway (typically the endoplasmic reticulum). The fact that Type III pathway "signals" are not removed from the mature protein means that they can be larger than the "temporary" signal peptides. Indeed, the Type III "signal" in the SopD2 protein is stated to be a sequence of 140-150 amino acids (page 37, Results, first paragraph) whereas a signal peptide for a protein undergoing vesicle-based secretion is typically up to 30-35 amino acids in length. As a result, it is submitted that the 140-150 amino acid targeting "signal" of SopD2 is not a signal peptide as is understood by the skilled person and so the claims are distinguished on this basis.

For completeness, it is pointed out that the reference in Brummel *et al.* to the ability of the SopD2 "signal" to target late endocytic compartments is a reference to the ability of this sequence to locate the SopD2 to its site of action in the infected host cell. It is not a reference any sort of vesicle packaging in the bacterial cell prior to release. Because the latter is the understood meaning of signal peptide in the present application, the reference in Brummel *et al.* to signalling does not relate to or disclose the present method.

Further distinction can be drawn when one looks at the final paragraph on page 38 of Brummel *et al.* where it states that control of the production of SopD2 is at the level of expression (and not at the post-translational level upon receipt of transient extracellular signals). Accordingly, for this reason also, this protein cannot be considered a bulk secreted protein as defined in the application and discussed further above. The Examiner is directed to the section of the description between the final paragraph of page 5 and the second paragraph on page 6 of the application as filed, which describes in detail the distinctions between control of protein secretion at the level of gene expression (e.g. SopD2) and control at the level of release (bulk secretion). Because Brummel *et al.* does not disclose a method that includes the elements of the claimed method, it does not anticipate the present claims. Thus withdrawal of this rejection is respectfully requested.

Claims 1, 2, 4-6, and 10-12 are rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Bryan (U.S. Patent 6,416,960 B1). In this regard the Office Action states the following:

> Bryan discloses the synthesis of conjugated proteins comprising Vargula hidgendorfi luciferase. The conjugated luciferase protein comprises the secretion signal of the ostracod secreted luciferase sequence. The luciferase can be produced using recombinant technology in mammalian host cells (see col. 25, lines 30-63).

The claims as amended all exclude the use of the entire sequence of a bulk secreted protein to drive secretion of a target protein. The conjugates of US 6,416,960 are designed to comprise the full length luciferase from *Vargula hilgendorfii* because the object of those conjugates is to use the luciferase as a molecular marker or at least a light emitting portion. Accordingly the conjugates of US 6,416,960 are distinct from the chimeric proteins of the claimed invention. Because Bryan does not disclose a method that includes the elements of the claimed method, it does not anticipate the present claims. Thus withdrawal of this rejection is respectfully requested.

Claim Objections

Claim 25 is objected for depending on a withdrawn claim.

Claim 25 is amended herein to refer to the host cells "as defined in claim 1" to avoid the need to recite the full description of the host cells of claim 1 in amended claim 25. This is believed to overcome this objection, and its withdrawal is respectfully requested.

A credit card payment is submitted herewith *via* EFS-Web in the amount of $490.00, which represents the large entity fee for a two-month extension of time under 37 C.F.R. § 1.17(a)(2). Applicant believes that this amount is correct; however, Applicant authorizes the Commissioner to charge to Deposit Account No. 14-0629 any additional fees that may be required, or to credit to the same account any overpayment of fees.

Respectfully submitted,

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